:

ANTIFUNGAL POTENCY OF SOME ANTIFUNGAL CHEMOTHERAPEUTICS AND ANTIDANDRUFF SOLUTIONS AGAINST DERMATOPHYTIC FLORA FROM LOCAL HAIRDRESSERS' TOOLS IN AWKA, NIGERIA

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ABSTRACT

Dermatophytes produce spores that can survive for a long time on fomites such as hairdressing tools (combs, scissors, clippers and hairpins). These fomites intend serve in the transmission of dermatophytosis. This study was aimed at evaluating and comparing the antifungal efficacy of some antifungal chemotherapeutics and antidandruff solutions against dermatophytic flora of hairdressers' tools in Eke-Awka Market in Awka, Anambra State, Nigeria. A total of forty-five (45) samples were collected by swabbing the hairdressing tools of the local hair dressers using sterile swab sticks.The antifungal drugs (Ketoconazole, Miconazole and Griseofulvin) and the antidandruff solutions (Shampoo and Conditioner) were also bought from Eke-Awka Market.The samples where inoculated into sterile Sabouraud Dextrose Broth base in test tubes and incubated at 25-27ºC for 5-14 days. After incubation, the test tubes with visually observed growth (turbidity) were subsequently plated out by streaking on freshly prepared Sabouraud Dextrose Agarsupplemented with Chloramphenicol (50µg/ml) and incubated at 25°C for up to 7-14 days. Identification was done using standard methods. Agar well diffusion method was employed for the *in vitro*antifungal susceptibility testing using different concentrations of both the antifungal drugs and antidandruff solutions obtained using double fold serial dilutions.Twenty Four (24) dermatophytes were isolated and identified.The isolates included *Microsporumferrugineum* 6 (25%), *Microsporum gypseum* 3 (12.5%), *Micosporum audouinii* 6 (25%), *Trichophytonschoenleinii* 5 (20.8%) and *Trichophyton mentagrophytes* 4 (16.7%).The percentage susceptibility pattern of the isolates showed that 60% of the isolates were susceptible to Ketoconazole, 40% respectively susceptible and susceptible doze dependent to Miconazole and 100% resistant to Griseofulvin. For the antidandruff solutions, 20% of the isolates were susceptible to hair shampoo while 100% were resistant to hair conditioner. The results of this study showed that dermatophytes are prevalent on the tools used by local hair dressers and also that the antifungal drugs had better activity on the isolates than the antidandruff solutions used.Also, based on this study, ketoconazole is recommended for dermatophytosis. There is therefore need to educate the populace on the health hazards associated with sharing local hair making tools and the need for clients to have their personal hair making tools.

Keywords: Dermatophytes, Antifungal, Antidandruff, Hair dressers, Tools, Awka.

1. INTRODUCTION

Hairdressing and hair maintenance are essential socio-cultural activities, particularly for women of African heritage. This is because hair had an important role in the culture of ancient African civilizations as it reflected one's family background, social rank, spirituality, tribe, and marital status [1]. Among varieties of tools utilized by Hairdressers, the hair brushes and combs are the most widely used in beauty salons. These tools can conceal and facilitate the spread of hair fungi flora from clients with diverse hair and scalp diseases. Consequently, there is a higher risk of infection when these fungi come into contact with another person's scalp. The majority of hair infections are eliminated under hostile environments, but

certain microbes, like dermatophytes, may adapt and endure. When constantly used without washing and drying, these hair brushes and combs at beauty salons usually gets moist, creating a favorable environment for the dermatophytes [2]. Because the hairdressers often use the same hairbrushes and combs on different clients at beauty salons without immediate effort to properly sterilize, the risk of increased dermatophytosis transmission [3]. It has been reported that high prevalence of dermatophytes contamination in West African hair dressing salon is due to poor socioeconomic status, particular hair style practices and genetic susceptibility [3].

Although dermatophyte infections frequently affect healthy people, those with weakened immune systems are more vulnerable. Many medications have been available for the treatment of superficial infections over the years including use of topical creams and herbal formulations. The type of therapy chosen depends on a number of factors, including the species involved, the location and severity of the fungal infection, and the safety, efficacy, and kinetics of the available drugs [4]. Oral antifungal medication is required, almost always, to remove dermatophytosis [5]. When given at the proper daily dosage and for the length of time required to achieve the desired results for each unique case, griseofulvin and terbinafine, is still a highly successful first line treatment for many Tinea infections caused by both Trichophyton spp. and Microsporum spp. [6]. However, the problems of long treatment durations, frequent treatment failures, resistance, unfavourable skin pharmacokinetic profile and poor oral bioavailability associated with the use of griseofulvin and other related antifungal agents has prompted the need for other alternative treatment options especially topical antifungals [7, 8].

Therefore, the purpose of the present study was to evaluate the i*nvitro* activities of selected antifungal drugs and commonly used topical antidandruff solutions against dermatophytic flora of local hairdressers' toolsin a local market in Awka, Anambra State, Nigeria.

2. MATERIAL AND METHODS

2.1 Study Area

The study Area is Eke-Awka Market which is the largest market in the town of Awka, capital of Anambra State, Nigeria. This market has a large number ofstalls and shops with very little spaces between them.

2.2 Sample Collection

Sample collection was preceded after an oral assent from the study participants. Samples were aseptically collected by swabbing the hairdressing tools (Combs of various sizes, scissors, hair pins, needles, etc.) of the local hair dressersusing sterile swab sticks. A total of Forty Five (45) samples were aseptically collected. The samples were transported to the laboratory in the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka without delay for mycological analysis. The samples were maintained in the refrigerator at 4^{o}C until they were ready to be analyzed.

The antifungal drugs (Ketoconazole, Miconazole and Griseofulvin) as well as the antidandruff solutions (Hair Shampoo and Hair Conditioner) used for this study were bought from Eke-Awka Market in Anambra State, Nigeria, stored under appropriate conditions until they were ready to be used.

2.3 Culture of Samples, Isolation and Identification

After aseptically collecting the samples with the sterile swab, samples where inoculated into sterile Sabouraud Dextrose Broth (SDA) base **(**Oxiod**,** UK**)**which has been prepared in test tubes according to the manufacturer's instruction and incubated at 25-27^{o}C for 5-14 days. After incubation, the test tubes where visually observed for growth (turbidity) and subsequently platted out by streaking on freshly prepared Sabouraud Dextrose Agar (Oxoid**,** UK) supplemented with Chloramphenicol (50μg/ml). The cultures where incubated aerobically at room temperature (25°C) for up to 7-14 days while observations were done daily. Positive cultures were examined according to [9]. In the absence of any growth after 4 weeks, the culture was considered negative. Pure colonies were stored on slants at 4^{o}C for further studies as described by [10]. The frequency of occurrence of the isolates was also recorded.

The dermatophytes were identified based on macroscopic appearance (colour, texture and pigmentation) and also microscopically by using the lactophenol cotton blue mount/slide culture method as described by [10]. The microscopic and colonial morphology were compared with photomicrographs and photomacrographs in the Colour Atlas by [11].

2.4Standardization of Inoculum and *In Vitro* Antifungal Susceptibility Testing using the Antifungal Agents and the Antidandruff Solutions

A representative of each of the species of dermatophytes isolated was used for the susceptibility testing. This implies five(5) microorganisms were used for the susceptibility testing. Ten-fold serial dilution was carried out on the test microorganisms which were previously grown on Sabouraud Dextrose Agar (SDA) for 4 days. Dermatophytes colonies were probed from SDA cultures. The tip of a sterile Pasteur pipette was used to obtain a mixture of mycelium and conidia which was mixed in 1ml distilled water in sterile tubes.The turbidity of the suspension was adjusted and then matched visually with 0.5 McFarland standards which is equivalent to 1 x 10^6 colony forming units per ml (CFU/ml). Swabs dipped into the inocula suspensions were streaked evenly over the surface of already prepared SDA (Oxoid, UK) plates. Wells of 8 mm in diameter each were dug into the SDA using a sterile cork borer.

Antifungal susceptibility testing was performed using double fold serial dilutions of three antifungal agents: ketoconazole, Miconazole and Griseofulvin. The agents were diluted to 50, 25, 12.5, 6.25, and 3.125 mg/ml. Then, the test agents at the different dilutions were pipetted (with the aid of micropipette) into the 8mm wells previously bored into the solidified agar, and the plates were incubated at 25°C for 3 to 7 days. After incubation, the size of the zones of inhibition around each well was measured and recorded. The test was conducted in duplicate and results presented as mean. Miconazole (50 µg) served as the Standard positive control against the *dermatophytes.* Criteria of susceptibility and resistance of antifungal agents were measured according to [12].

The commercially available shampoo (Vinox shampoo-coded as VS) and hair conditioner (Style Plus hair conditioner-coded as SC) were diluted with sterile distilled water to get different double fold dilutionsof 50, 25, 12.5, 6.25, and 3.125ml. The antifungal assay with these dilutions was carried out as described for the antifungal drugs above. After incubation, the inhibition zone diameter was measured and recorded according to CLSI guidelines. The test was conducted in duplicate and results presented as mean.

3. RESULTS AND DISCUSSION

3.1 Occurrence of the Dermatophytes

A total of Twenty Four (24) isolates of dermatophytes was identified by macroscopic and microscopic morphological characteristics. The isolates belonged to two genera*: Microsporum* (15 cases)—the most prevalent cause of dermatophytosis in this study, accounting for 62.5% of isolates—and *Trichophyton* (9 cases; 37.5%). The isolates included *Microsporumferrugineum* 6 (25%), *Microsporum gypseum* 3 (12.5%), *Micosporum audouinii* 6 (25%), *Trichophytonschoenleinii* 5 (20.8%) and *Trichophyton mentagrophytes* 4 (16.7%). *M.ferrugineum and M. audouinii*have the highest percentage of occurrence of 25% each as against *Microsporum gypseum* with the least percentage occurrence of 12.5% (table 1).This result is similar to that of [3] who isolated thirty (30) dermatophytes from hairdressing tools of some selected hairdressing salons in Bamako, Mali. The isolates also belong to the two genera *Microsporum* and *Trichophyton* just as in the present study. Also, results of the present study agree with the work of [13] who found *M. audouinii* to be associated with combs and scissors as one of the causative agents of dermatophytosis. It is an evidence of presence and transmission of the disease among clients. However, the results of this study is not in agreement with the works of [14]and [15]who stated in their findings that *Trichophyton* was the most common genus isolated*.* The findings of this study also contradicts that of [16], who reported that *Microsporum canis* was the most common isolate, followed by *Microsporum. Gypseum,T. mentagrophytes* and finally*Microsporum audouinii*. The differences in the frequencies and genera of microorganisms isolated between this study and previous studies may likely stem from variations in the environments.

Table 1: Occurrence of the Isolated Dermatophytes

Microorganism	Frequency	Percentage(%)
Microsporumferrugineum	*6*	*25*

Microsporum gypseum	*3*	*12.5*
Microsporum audouinii	*6*	*25*
Trichophytonschoenleinii	*5*	*20.8*
Trichophyton mentagrophytes	*4*	*16.7*
Total	**24**	**100**

3.2 *In Vitro* Antifungal Susceptibility Testing Using the Antifungal Agents

The results of the susceptibility profile of the isolates to the antifungal agents are presented in Table 2. It can be observed from the table that ketoconazole was the most effective as 60% of theisolated organisms were susceptible to it while 40% were susceptible dose dependent. Griseofulvin had the poorest activity as all the microorganisms (100%)were resistant to it.The result of this study is similar to that of [17] who reported that dermatophytes were most susceptible to ketoconazole among other antifungal drugs tested, thus suggesting that ketoconazole can be used to treat a majority of dermattophytic infections. Some other studies[15,18] reported that griseofulvin was the leastactive antifungal drug against *T. mentagrophytes* isolates which agrees with the result of the present study.Also, [19] reported in their findings that griseofulvin was the least effective (after fluconazole) against some isolated dermatophytes, including *Trichophyton mentagrophytes*.As shown in table 5, even at the highest concentration of 50mg/ml for griseofulvin, the values of the inhibition zone diameters against all the tested dermatophytes were very low for any of them to be considered as susceptible, according to Clinical Laboratory Standard Institute (CLSI) standards. However, the efficacy of griseofulvin can be enhanced by nanotechnology. A study by [20] showed that nanoparticles of griseofulvin with zinc oxide had effective inhibitory action against *Trichophyton mentagrophytes* and *Trichophyton verrucusom.*

The susceptibility pattern of each isolate to different concentrations of each drug indicating the inhibition zone diameter (IZD) is shown in Tables 3, 4 and 5 as well as in figure 1.

Table 2: Antifungal Susceptibility Pattern of the Isolated Dermatophytes Species to the Antifungal Agents

Antifungal Agents	S (%)	SDD (%)	R (%)
Ketoconazole	**3 (60)**	**3 (40)**	**0 (0)**
Miconazole	**2 (40)**	**2 (40)**	**1 (20)**
Griseofulvin	**0 (0)**	**0 (0)**	**5 (100)**

Key: S = Susceptible; **SDD=** Susceptible Dose Dependent; **R =** Resistant

Table 3: Inhibition Zone Diameters of the Different Concentrations of Ketoconazole against theIsolated Dermatophytes

	Concentration (mg/ml) / Inhibition Zone Diameters (mm)					
Microorganisms	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.562**
Microsporumferrugineum	27	25.5	24.5	24.5	21	19
Microsporum audouinii	27	27	27	25.5	23.5	22
Microsporum gypseum	15	15	14	12	12	10
Trichophyton mentagrophytes	27	24	22.5	21	20	17
Trichophytonschoenleinii	14	14	13	12	11	10

Table 4: Inhibition Zone Diameters of the Different Concentrations of Miconazole against theIsolated Dermatophytes

	Concentration (mg/ml) / Inhibition Zone Diameters (mm)					
Microorganisms	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.562**
Microsporumferrugineum	21 20	20 18	18 15			
Microsporum audouinii	22 20	19 19	17 16			
Microsporum gypseum	10.5 11	10 8	5.5 4			
Trichophyton mentagrophytes	13 13.5	13 12	10 8			
Trichophytonschoenleinii	15.5	14.5 14	13 12 10			

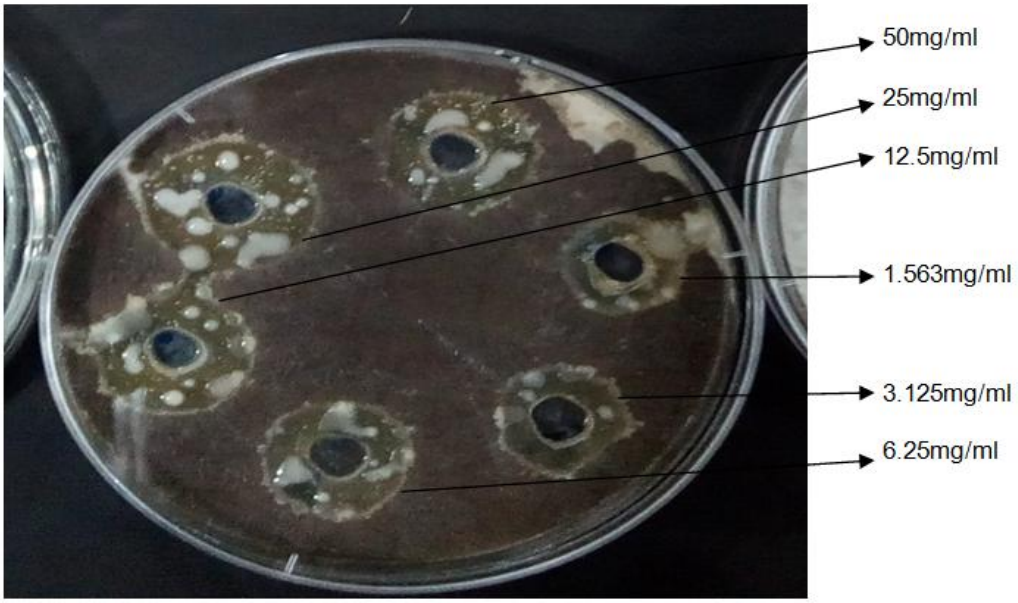

Fig. 1 Inhibition Zone Diameters of the Different Concentrations of Miconazole against *Trichophyton mentagrophytes*

Table 5: Inhibition Zone Diameters of the Different Concentrations of Griseofulvin against theIsolated Dermatophytes

	Concentration (mg/ml) / Inhibition Zone Diameters (mm)					
Microorganisms	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.562**
Microsporumferrugineum	7 5.5	4.5 4.5	2 0			
Microsporum audouinii	4 2	2 00	0			
Microsporum gypseum	5 1	0 00	0			
Trichophyton mentagrophytes	3 2	2 10	0			
Trichophytonschoenleinii	4 1	3 30	0			

3.3*In Vitro* Antifungal Susceptibility Testing Using the Antidandruff Solutions

Table 6 shows the results of the susceptibility profile of the isolates to the antidandruff solutions. It can be seen from the table that the microorganisms showed 20% susceptibility and were also 20% susceptible dose dependent to shampoo which made it more effective than hair conditioner to which all the five (5) representative microorganisms were resistant (100%).

The susceptibility pattern of each isolate to the different concentrations of each antidandruff solutionindicating the inhibition zone diameter (IZD) is shown in Tables 7 and 8 as well as in figures 2 and 3. This result is in contrast with the findings of [18] in which the antidandruff conditioner and antidandruff shampoo used were effective against *Trichophyton mentagrophytes*with larger inhibition zone diameters.A possible explanation for this difference may be attributed to a reduction in the concentration of the active ingredientsduring the double fold dilution [21].Also, apart from the concentrations of the solutions used,the little or no activity of the antidandruff solutions (hair conditioner and shampoo)used in this study on the isolates could be attributed to the brand of the antidandruff solutions used as another brand may yield a positive result.

Table 6: Antifungal Susceptibility Pattern of the Isolated Dermatophytes Species to the AntidandruffSolutions

Antifungal Agents	S (%)	SDD (%)	R (%)
Shampoo	**1 (20)**	**1 (20)**	**3 (60)**
Hair Conditioner	**0 (0)**	**0 (0)**	**5 (100)**

Key: S = Susceptible; **SDD=** Susceptible Dose Dependent; **R =** Resistant

Table 7: Inhibition Zone Diameters of the Different Concentrations of Shampoo against theIsolated Dermatophytes

	Concentration (mg/ml) / Inhibition Zone Diameters (mm)					
Microorganisms	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.562**
Microsporumferrugineum	5.5	5.5	4.5	4	1	0
Microsporum audouinii	15.5	15	14	13	13	9
Microsporum gypseum	27	22	21	20	19	11
Trichophyton mentagrophytes	7	7.5	7	30	0	
Trichophytonschoenleinii	7	9	3	0	0	0

Table 8: Inhibition Zone Diameters of the Different Concentrations of Conditioner against theIsolated Dermatophytes

	Concentration (mg/ml) / Inhibition Zone Diameters (mm)					
Microorganisms	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.562**
Microsporumferrugineum	3	3	0	0	0	0
Microsporum audouinii	2	0	0	0	0	0
Microsporum gypseum	1	0	0	0	0	0
Trichophyton mentagrophytes	4	3	2	0	0	0
Trichophytonschoenleinii	1	0	0	0	0	0

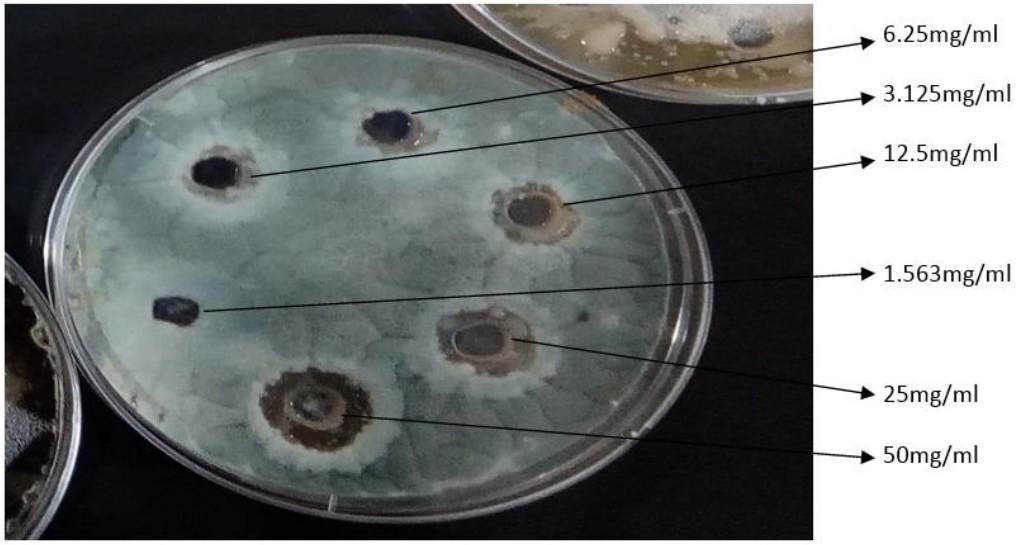

Fig. 2 Inhibition Zone Diameters of the Different Concentrations of Shampoo against *Microsporum ferrugineum*

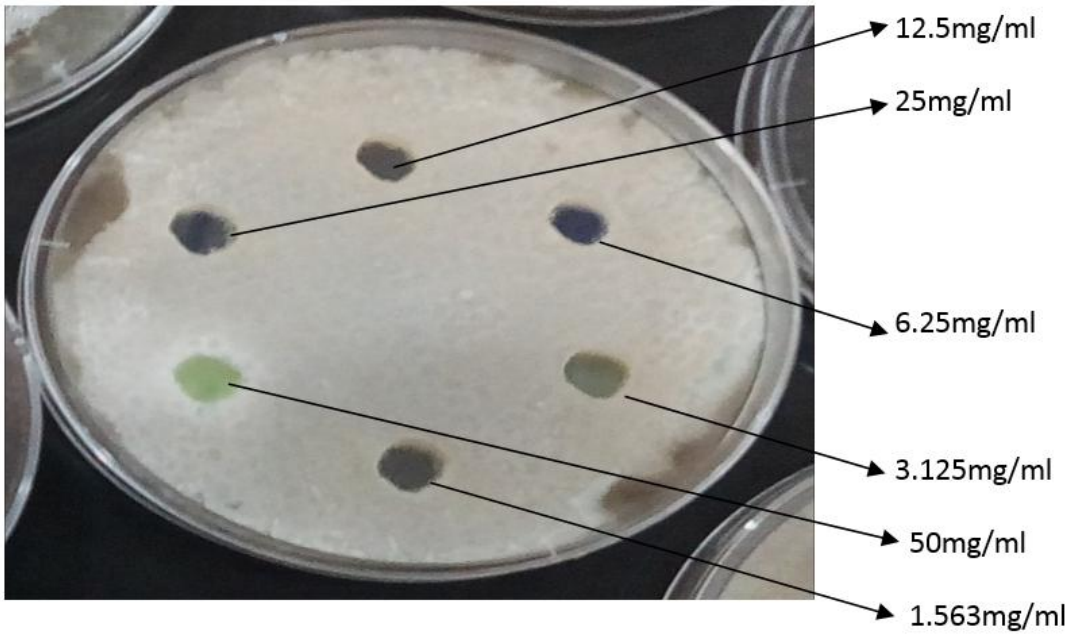

Fig 3: Total Resistance (No Inhibition Zone Diameters) of *Trichophyton schoenleinii* against Hair Conditioner

4. CONCLUSION

In this study,*Microsporumferrugineum* and *Micosporum audouinii*were the most isolated dermatophytes with each having a prevalence of 25%. Ketoconazole was the most effective among the antifungal drugs as 60% of the isolates were susceptible to it.All the dermatophytes (100%)were resistant to Griseofulvin while 40% were respectively susceptible and susceptible doze dependent to Miconazole. For the antidandruff solutions, 20% of the isolates were susceptible to hair shampoo while all were resistant to hair conditioner. The results of this study showed that dermatophytes are prevalent on the tools used by local hair dressersin the study area and also that the antifungal drugs had better activity on the isolates than the antidandruff solutions used.Also, based on this study, ketoconazole is recommended for dermatophytosis. There is therefore need to educate the populace on the health hazards associated with sharing local hair making tools and the need for clients to have their personal hair making tools.

REFERENCES

1.Omotoso SA.Gender and hair politics: An African philosophical analysis. Africology: The Journal of Pan African Studies. 2018;12(8):5-19.
2. Edward SM, Megantara I, Dwiyana RF.Detection of fungi in hair-brushes in beauty salons at Jatinangor. Althea Medical Journal. 2015;2(4): 516-520.
3. Coulibaly O, Thera MA, Piarroux R,Doumbo OK., Ranque S. High dermatophyte contamination levels in hairdressing salons of a West African suburban community. Mycoses. 2015;58(2): 65-68.
4. Bellmann R, Smuszkiewicz P. Pharmacokinetics of antifungal drugs: practical implications for optimized treatment of patients. Infection. 2017;45: 737-779.
5. Brescini L, Fioriti S, Morroni G, Barchiesi F. Antifungal combinations in dermatophytes. Journal of Fungi. 2021;7(9): 727.
6. Alter SJ, McDonald MB, Schloemer J, Simon R, Trevino J. Common child and adolescent cutaneous infestations and fungal infections. Current problems in pediatric and adolescent health care. 2018;48(1): 3-25.
7. Durdu M, Ilkit M, Tamadon Y, Tolooe A, Rafati H, Seyedmousavi S. Topical and systemic antifungals in dermatology practice. Expert review of clinical pharmacology. 2017;10(2): 225-237.
8. Khurana A, Sardana K, Chowdhary A. Antifungal resistance in dermatophytes: Recent trends and therapeutic implications. Fungal Genetics and Biology.132: 103255.
9. Ogbo FC. Basic Microbiology: Fundamentals and Techniques. Cresco Printing and Publishers Enugu; 2005. pp 12-15.
10. Cheesbrough M. Distinct Laboratory Practical in Tropical Countries, Part 2. Cambridge University Press, UK; 2006.

11. Frey D, Oilfield RJ, Bridger RC. A Colour Atlas of Pathogenic Fungi. Wolfe Medical Publications Ltd; 1979. pp. 71 – 93.
12. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic Susceptibility Testing by Standardized Single Method. American Journal of Clinical Pathology. 1996;45 (4):493-496.
13. Chidi-Onuorah LC, Oyeka CA, Kyrian-Ogbonna EA, Ezeadila JO. Screening of Some Antifungal Drugs on Fungal Isolates from Barbering Equipment.Nigerian Journal ofMicrobiology. 2017; 31(2): 4054-4058.
14. Jha BK, Murthy SM, Devi NL. 2012. Molecular Identification of Dermatophytosis by PolymeraseChain Reaction (PCR) and Detection of Source of Infection by Restricted Fragment Length Polymorphism (RFLP). Journal of College of Medical Sciences-Nepal. 8(4):7–15.
15. Kumar MB, Gulati N, Chander J, Singla N, Bhalla M, CharuNayyar C, et al. Species Distribution and Antifungal Susceptibility Profile of Dermatophytes from a Tertiary Care Centre in North India. Journal of Laboratory Physicians. 2022; 7;14(4):449-455.
16. Shalaby M, AmsaaNE, Mohammed AE. Isolation, identification and *Invitro* Antifungal Susceptibility Testing of Dermatophytes from Clinical Samples. Indo American Journal of Pharmaceutical Research. 2016; 3 (2):227-231.
17. Satyendra KS, Dheeraj KP, Ragini T, Arghya D, Tej BS.*In vitro* susceptibility of dermatophytes to oral antifungal drugs and amphotericin B in Uttar Pradesh, India. Indian Journal of Dermatology, Venereology and Leprology. 2019; 85(4):388-392.
18. Gülgün Y, Ebru T, Aydan Y, Yunus B.*In vitro* activities of antifungal drugs against dermatophytes isolated in Tokat, Turkey. International Journal of Dermatology. 2013; 52(12):1557-1560.
19. Badiee P, Shokohi T, Hashemi J, Mohammadi R, Najafzadeh MJ, Shahidi MA, et al. Comparison of *in vitro* activities of newer triazoles and classic antifungal agents against dermatophyte species isolated
from Iranian University Hospitals: a multi‐central study. Annals of Clinical Microbiology and Antimicrobials. 2023; 22:15.
20. Hussein AA, Janabi AL, Bashi AM. Synthesis and antifungal activity of novel griseofulvin nanoparticles with zinc oxide against dermatophytic fungi: *Trichophytonmentagrophytes* and *Trichophytonverrucosum:* A primary study.Current Medical Mycology. 2022; 8(2): 40- 44
21. dos Santos RM, Dias-Souza MV.. Effectiveness of five antidandruff cosmetic formulations against planktonic cells and biofilms of dermatophytes. Saudi Journal of Biological Sciences. 2017; 24(2):331-337.